Diversity in Mechanisms of Endothelium-Dependent Vasodilation in Health and Disease

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Abstract

Small arterioles (40–150 μm) contribute to the majority of vascular resistance within organs and tissues. Under resting conditions, the basal tone of these vessels is determined by a delicate balance between vasodilator and vasoconstrictor influences. Cardiovascular homeostasis and regional tissue perfusion is largely a function of the ability of these small blood vessels to constrict or dilate in response to the changing metabolic demands of specific tissues. The endothelial cell layer of these microvessels is a key modulator of vasodilation through the synthesis and release of vasoactive substances. Beyond their vasomotor properties, these compounds importantly modulate vascular cell proliferation, inflammation, and thrombosis. Thus the balance between local regulation of vascular tone and vascular pathophysiology can vary depending upon which factors are released from the endothelium. This review will focus on the dynamic nature of the endothelial released dilator factors depending on species, anatomic site, and presence of disease, with a focus on the human coronary microcirculation. Knowledge how endothelial signaling changes with disease may provide insights into the early stages of developing vascular inflammation and atherosclerosis, or related vascular pathologies.

Introduction

There are a number of vasoactive substances produced by endothelial cells that elicit vasodilation. The prototype is nitric oxide (NO), made by the constitutively expressed enzyme nitric oxide synthase (NOS). NO is synthesized in the endothelial cell layer and signals to underlying vascular smooth muscle cells (VSMCs), where it elicits hyperpolarization and relaxation primarily in a cyclic GMP-dependent manner (Figure 1). A second major family of endothelial derived vasodilator substances are prostaglandins, with the classic example being prostacyclin. Prostaglandins are also constitutively generated by the action of cyclooxygenase (COX) enzymes on arachidonic acid. They traverse the intercellular space and elicit a cAMP-dependent hyperpolarization of VSMCs (Figure 1). The third family of vasoactive substances is generally referred to as endothelial-derived hyperpolarizing factors (EDHFs). EDHFs ultimately cause vasodilation by hyperpolarizing VSMCs via stimulation of K^+ channels or Na^+/K^+- ATPase. Multiple EDHFs have been described, including hydrogen peroxide (H_2O_2), epoxyeicosatrienoic acids (EETs), carbon monoxide (CO), hydrogen sulfide (H_2S), C-natriuretic peptide (CNP), anandamide, and the potassium ion itself [17].

Studies in animals reveal a rich diversity of vasoactive signaling pathways depending on the vessel size, vascular bed, species, and health of the organism being studied. The purpose of this review is to emphasize the extent of this diversity, focusing on the coronary

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microcirculation where possible (vessels <150 μm in diameter), and highlighting the unique vasomotor plasticity attendant within the human microcirculation in health and disease. While it could be argued that some of the observed diversity among such a large cross-section of studies is dependent on the method of study (for example *in vivo* vs. *in vitro* vessel preparations), it is worth noting that the majority of the studies highlighted in this review use standard cannulated vessel preparations to isolate direct vascular responses in the microvasculature from humoral, neural, and other indirect influences [77].

Diversity and Endothelial Dependent Dilator Mechanisms in Animals

Animal studies have shown that the small coronary arterioles regulate the majority of vascular resistance in the heart [51], therefore modulation of vascular tone in these arterioles is under tight metabolic, myogenic and humoral control [57]. However, the mechanism of endothelium-dependent dilation can vary across species, or even within the same species, depending on the conditions. Consider the stimulus of shear stress produced by an increase in flow through a vessel. The resulting mechanical force activates an endothelium-dependent vasodilation in most vascular beds. Kuo et al. showed that flow-mediated dilation (FMD) in porcine coronary arterioles is completely abolished by inhibiting NOS with L-nitroarginine methyl ester (L-NAME), indicating an exclusive role for NO in this response [40]. Similarly, studies in guinea pig coronary resistance arterioles [80], rat mesenteric arterioles [46], and rat intracerebral arterioles [64] reveal NO as the primary mediator of flow-mediated dilation, while prostaglandins contribute little to the response. Conversely, in small arterioles of the rat cremaster muscle *in vivo*, flow-mediated dilation is primarily mediated by prostaglandins with little role for nitric oxide [38]. However co-release of both nitric oxide and prostaglandins contribute to flow-mediated vasodilation in isolated rat gracilis muscle arterioles [37]. In female endothelial nitric oxide synthase (eNOS) knockout mice, flow-mediated dilation in skeletal muscle arterioles is exclusively mediated by an EDHF (epoxyeicosatrienoic acid) derived from arachidonic acid [29].

Similar to shear stress, a wide range of signaling mechanisms have been reported for agonist-induced vasodilation. For example, adenosine is a potent metabolite dilator of arterioles in multiple vascular beds, however the mechanism is species-specific. Adenosine dilates isolated coronary arterioles from adult pigs [27] and guinea pigs [81] in an endothelium-dependent, NO-mediated manner. Similar responses have also been reported in the conductance coronary vessels of conscious dogs [68]. Conversely, in both main trunk and branch circumflex bovine arterial strips adenosine dilation requires activation of cAMP, suggesting that NO may not play a prominent role in this species at all [78]. Meanwhile, other species show either a partial direct vasodilator effect of adenosine [39, 75], or a vasodilator response that is independent of the endothelium altogether [35, 84].

Bradykinin (BK) is a potent and frequently studied endothelium-dependent dilator of multiple vascular beds, and similar to studies that have examined the mechanisms of adenosine-mediated vasodilation, the mediators of BK-induced dilation are also highly dependent on species and vascular bed. In the cerebral circulation of the rat, BK is a potent NO-dependent vasodilator of isolated middle cerebral arteries [24], but in 3rd order arterioles of the cremaster muscle in the same species, dilation is completely independent of NO [1]. The coronary circulation of different mammalian species also shows a wide diversity of dilator mechanisms to BK. In the rat, dilation is completely independent of NO and prostaglandins, and appears to be mediated by an EDHF derived from a metabolite of arachidonic acid [21, 23], while in the rabbit both NO and prostaglandins play a role [42]. In isolated epicardial right coronary arteries from the porcine coronary circulation, dilation to bradykinin is insensitive to both L-NAME and indomethacin, but is completely abolished in the presence of catalase, indicating a key role for H_2O_2 as the EDHF responsible for BK-

induced vasodilation [52]. This is further supported by a study conducted by Payne et al., which showed that BK-induced vasodilation is significantly reduced in isolated left circumflex coronary arteries of dogs in the presence of TEMPOL, an antioxidant [69].

Mechanism(s) of human microvascular endothelium-dependent dilation

The human microcirculation exhibits developmental patterns of endothelium-dependent dilation similar to those observed in animals, but with several unique differences. In animal models, in infancy, prostaglandins are primarily responsible for acetylcholine-dependent vasodilation (vertebral arteries), while in adult animals the same vascular bed relies on NO as the mediator of acetylcholine-mediated dilation [11]. In humans, plasma concentrations of prostacyclin (PGI_2), the primary endothelium-derived prostaglandin vasodilator, peak at birth and then decrease throughout life [32]. In preliminary studies we have observed that flow-mediated vasodilation in isolated coronary microvessels of infants is exclusively mediated by prostaglandins (cyclooxygenase dependent) [86]. In early adulthood, both NO and prostaglandins contribute, while later in adulthood, NO predominates as the primary factor responsible for FMD [86]. The situation changes dramatically with the onset of disease. For example, in adults with coronary artery disease undergoing bypass surgery, FMD in isolated atrial arterioles is slightly reduced but is NOS- and prostaglandin-independent, being mediated by H_2O_2 as discussed in more detail below [55].

Similar to other mammalian species, diversity exists across human vascular beds in endothelium-dependent dilator responses. In isolated microvessels from subcutaneous adipose tissue, NO and prostaglandins play no role in the dilation to bradykinin, while in visceral adipose arterioles, sensitivity to bradykinin is markedly reduced in the presence of NOS inhibition [76]. Gender and age were also shown to importantly modulate these endothelium-dependent responses [76].

Effect of Genetic Manipulation and Disease on Endothelium- Dependent Dilation

The diversity of endothelium-dependent vasodilator mechanisms present in normal mammalian blood vessels broadens when normal physiology is perturbed with genetic manipulation or disease, revealing a complex compensatory or alternative response system to maintain vasodilation. For example, in wild type mice the vasodilator response to acetylcholine is primarily dependent on NO derived from eNOS in the small coronary arteries [43], while the response to shear is approximately 50% inhibitable by L-NAME [30] in the same vascular bed. Interestingly, in eNOS knockout (KO) mice, the magnitude of the vasodilator response to both acetylcholine and shear stress is no different than in wild type mice [30, 44]. The response remains NO-dependent, however the enzyme responsible for the production of NO shifts to the neuronal isoform of NOS (nNOS), which is normally localized in the intracardiac neurons lining the coronary vessels [34]. Under normal conditions nNOS does not contribute to NO-dependent vasodilation, but with loss of eNOS, the signaling mechanisms in response to both flow and acetylcholine co-opt the nNOS pathway to maintain stimulus-induced NO production and dilation. This artificially induced pathological state is mimicked by pathological states as seen in vessels from spontaneously hypertensive rats [4] and humans with atherosclerosis [85], where significant upregulation of nNOS suggests that a similar compensatory mechanism may be operative.

Vasodilation to bradykinin has also been shown to persist in the coronary circulation of eNOS deficient animals; however the mediator of dilation switches from NO to a CYP450-derived EDHF in the KO animals [14]. While vasodilation has been shown to persist in the eNOS-specific KO mice, it is worth noting that in the double and triple NOS knockout

animals there is progressive loss of acetylcholine-induced dilation as this compensation is lost [59].

It has been widely demonstrated that an association exists between the presence of coronary artery disease (CAD), or its risk factors, and an elevation in vascular superoxide production. Superoxide is a short-lived free-radical, but it reacts 3 times more rapidly with NO to form peroxynitrite than it is dismutated by the superoxide dismutase enzymes, causing a net reduction in the bioavailabilty of NO [3, 13, 16]. Peroxynitrite and the superoxide anion can also oxidize BH_4 (a critical cofactor of eNOS) to BH_2, uncoupling eNOS so that it no longer produces NO. As with genetic depletion of the eNOS enzyme, disease-induced elevations in superoxide also create an environment where NO bioavailability is reduced [26, 33, 45, 60, 73, 83]. This situation invokes a degree of vasodilator plasticity, much like that observed in knockout mice lacking the eNOS enzyme. This disease-induced compensation is best exemplified in a study by Najibi et al. who examined dilation to acetylcholine in isolated carotid artery segments from hypercholesterolemic rabbits. After 10 weeks of dietary hypercholesterolemia, the sensitivity and magnitude of dilation were identical between the normal and hypercholesterolemic animals [58]. However, the mechanism had changed from one that was primarily mediated by NO-cGMP to one that was instead mediated by an unidentified EDHF. Similarly, Clark and Fuchs reported that the degree of vasodilation to acetylcholine was similar in isolated small coronary arteries from both healthy and cardiomyopathic Gold Syrian hamsters; however the mediator of vasodilation switched from NO in control animals to a cyclooxygenase-derived EDHF in animals with cardiomyopathy [12].

How are these compensatory pathways invoked, often rapidly? One hypothesis to explain these observations is that under normal conditions NO may exert negative feedback to mask these compensatory vasodilator pathways [66]. For this hypothesis to be correct, one would expect exogenous application of NO to have a similar effect. In support of this contention, Nishikawa et al. showed that dilation of canine coronary microvessels to BK is mediated by EDHF *in vivo* when endothelial nitric oxide synthase and cyclooxygenase are inhibited with L-NAME and indomethacin, respectively [66]. In this situation, co-administration of a low, non-dilator dose of the NO donor sodium nitroprusside completely abolished the EDHF-mediated dilation to BK, implicating a role for endogenous NO in inhibiting the compensatory vasodilator pathways under non-disease situations. Similar findings have also been reported in isolated carotid artery segments of rabbits and in porcine coronary arterioles [2]. However not all studies show such a modulating effect of NO [18].

Human microvascular reactivity during stress and disease

As described above, in the presence of CAD, the mechanism of human coronary resistance vessel dilation to shear stress changes from NO to EDHF [54]. Further studies have shown us much about this changed signaling pathway, linking shear stress to mitochondrial production of EDHF. We have also explored the nature of the EDHF involved, and the stimulus activating smooth muscle hyperpolarization.

As demonstrated by bioassay, flow-mediated dilation of isolated human coronary arterioles from patients with CAD elicits release of a transferrable factor that stimulates large conductance calcium-activated potassium channels in the underlying vascular smooth muscle [47]. This factor is hydrogen peroxide, derived from endothelial mitochondria [48, 54]. Interestingly, cytochrome P450 monooxygenase products, likely epoxyeicosatrienoic acids, are also necessary for the flow-mediated dilation [55] but appear not to be the transferrable agent acting on vascular smooth muscle (unpublished observations demonstrating lack of endothelial release of EET in response to shear). In isolated arterioles

from human visceral adipose, a transition is also seen from NO to H_2O_2 as the primary mediator of FMD with the onset of CAD [72]. Thus adipose microvessels might serve as a surrogate for disease-induced changes in coronary vascular reactivity. (Figure 2)

Other disease states may also evoke release of alternative endothelium-mediated dilators in the human microcirculation. In patients with diabetes there is upregulation of COX-2 expression and greater participation by prostaglandins through COX-2 in coronary arteriolar dilation to bradykinin [79]. One of the most striking compensatory responses is observed in isolated mucosal arterioles of patients with inflammatory bowel disease (IBD) [13]. In adjacent unaffected bowel, dilation to acetylcholine is mediated by endothelial release of NO. However in affected tissue, a slightly reduced dilation is seen, but the response is mediated entirely by prostaglandin D2 through an endothelium-independent mechanism likely involving the underlying smooth muscle [25]. (Figure 2)

Mechanism of endothelium shear signal transduction

How is shear transduced by human coronary endothelial cells to cause mitochondrial production of reactive oxygen species? Recent studies from Zhang's laboratory indicate that lipid raft-bound mechanosensitive transient receptor potential vannilloid 4 (TRPV4) channels are critically involved in human coronary arteriolar FMD [5], and activation of these channels is linked to production of hydrogen peroxide in a mitochondrial-dependent fashion [5].

Alternatively shear may activate calcium entry by other mechanisms. The elevated cell calcium can stimulate phospholipases, cleaving arachidonic acid from cell membranes. Constitutively expressed cytochrome P450 acts on the free arachidonic acid to produce EETs [19]. EETs are known to activate TRPV4 channels and this could explain both their critical role in FMD and their lack of release into the extracellular space [50]. Studies are underway to discern the direct and/or indirect pathways by which shear activates TRPV4 leading to coronary dilation.

Superoxide produced by the mitochondria in response to shear is converted to hydrogen peroxide by MnSOD in the mitochondrial matrix or by CuZn SOD in the intermembrane space. H_2O_2 is freely permeable and can exit both mitochondrial and plasma membranes and enter the underlying smooth muscle cell. There are a variety of ways that H_2O_2 can elicit dilation, including activation of PI3K [9], NOS [15], Kv channels [74], guanylyl cyclase [7] or by opening large conductance calcium-activated potassium (BKca) channels directly [53], or through activation of PKG1α [6]. In human coronary arterioles the dilation involves activation of PKG1α, likely through oxidation of the CYS42 residue, forming a disulfide dimer between two molecules of the enzyme [47]. A summary of the proposed signaling pathways responsible for FMD in human coronary arterioles are shown in Figure 3.

Time-Course of Compensatory Dilator Pathways

It is clear that a transition occurs from NO to H_2O_2 as the mediator of human coronary (and adipose) arteriolar dilation in the presence of CAD, but it is not known how rapidly this transition occurs. To examine this question we have subjected healthy individuals to a brief hypertensive stress during isometric exercise [31, 71]. The modest elevation in blood pressure impaired conduit endothelial function (brachial artery flow-mediated dilation) in sedentary subjects [31, 71]. However when athletes were tested, no change in endothelial function was observed. We then extended these findings to the microcirculation using adipose arterioles from gluteal fad pad biopsies of subjects undergoing exercise. Preliminary observations indicate reduced FMD in sedentary subjects following the acute exercise-induced hypertension, however in athletes a different response was observed [70]. FMD in

post-exercise arterioles was similar to that observed in pre-exercise vessels, but the mechanism of dilation had shifted from NO to H_2O_2, similar to that observed in coronary arterioles [70]. These preliminary findings suggest that the transition in mechanism of dilation can occur rapidly, even within an hour, and that the switch in mediators of dilation is similar in athletes and patients with chronic coronary artery disease.

Consequences of Multiple Dilator Pathways

The wide range of vasodilator mechanisms observed in different animal species raises questions about the rationale for the evolutionary diversity of mechanisms of endothelium-dependent dilation. The answers might relate to the non-vasomotor properties of these varied mediators. For example, nitric oxide is a potent inhibitor of platelet and leukocyte adhesion, smooth muscle proliferation and migration, and oxidative stress [20, 28, 49, 61–63]. Therefore, as a common physiological product of healthy endothelial cells, NO is intimately involved in preventing vascular inflammation and atherosclerosis. Decades of research in both animals and humans have shown that risk factors for coronary artery disease up regulate vascular superoxide production, which both quenches and inhibits the production of NO, even though endothelium-dependent dilation may still be present, as has been reported in the rabbit carotid artery [58]. Conversely, vasodilation may be absent as Kuo and colleagues reported in the coronary arterioles of hypercholesterolemic pigs [41], or dilation may persist but be mediated by a different endothelium-derived dilator altogether, such as hydrogen peroxide as seen in arterioles isolated from human adipose tissue [72] or epoxyeicosatrienoic acid as observed in bovine coronary arterioles [10]. It is important to consider that although both H_2O_2 and EETs produce dilation, they have very different effects on atherogenic potential. While EETs share many of the atheroprotective features of NO [65, 82], hydrogen peroxide promotes a pro-inflammatory and pro-atherosclerotic phenotype [8, 22, 56]. It remains to be seen if compensatory H_2O_2-mediated vasodilation, as is observed in the human coronary circulation of patients with CAD [54], acts as a feed-forward mechanism to further promote an atherogenic phenotype.

As described above, this shift in mediator is also observed in preliminary studies in healthy athletes who maintain endothelium-dependent dilation following brief (hypertensive) stress by converting from NO as the endothelial mediator to H_2O_2 [70]. There are several plausible explanations for this transition in endothelial function. Perhaps the ability to continuously maintain endothelium-dependent dilation is of greater importance than the risk of short-term vascular oxidative stress during heightened production of H_2O_2. It is conceivable that the energy required to produce H_2O_2 in a stressed environment is more favorable than the process required to generate NO in such an oxidative environment. H_2O_2 is a more stable mediator of dilation in disease than is NO, because the latter is readily quenched by elevated ambient levels of superoxide. Another explanation is that dismutation of superoxide to H_2O_2 circumvents generation of peroxynitrite, which may be even more detrimental in terms of vascular damage.

It is unclear why such a transition is seen both during health and disease, prompting consideration about the type and duration of stress that is necessary to promote vascular pathology. For example, are there functional differences between short vs. longer term production of H_2O_2 in lieu of NO? There may be lessons learned from other species as well. For example, the pig, which is highly susceptible to coronary artery disease, loses NO as a coronary endothelium released product during hypercholesterolemia [41]. However, the dog, in which induction of coronary plaques is notoriously difficult, has been shown to maintain NO-dependent vasodilation in the coronary arteries when fed a high fat diet [36], and demonstrates a prominent dilation to EETs in the coronary microcirculation under normal conditions [67]. It is worth noting that EETs have an atheroprotective profile similar to NO

[65, 82]. Thus, the consequences of unmasking alternative vasodilator pathways should be considered as potentially harmful rather than as strictly a compensatory "second line of defense" in the vasculature [66].

Summary and Conclusions

The diversity in vasodilator mechanisms across species and vascular beds is striking both in health and disease. Bioavailability of nitric oxide, the prototypical mediator of endothelium-dependent dilation, is highly sensitive to the tissue redox state. It has widely been shown that brief oxidative stress associated with exercise or the chronic stress of disease can switch off NO production. In some cases, such as in the pig coronary arteriole, this results in a loss of endothelium-dependent dilation (to increased shear). However, in others (human coronary and adipose microcirculations) compensatory dilation is unveiled with mitochondrial-derived hydrogen peroxide acting as the principal vasoactive mediator. This has the potential to disrupt the tonic inhibitory endothelial influence on smooth muscle proliferation and vascular inflammation, and may result in a pro-atherosclerotic environment over time. Further investigation is needed to better understand the signaling pathways that are responsible for the shift in mediators of endothelium-dependent dilation. This could lead to new approaches to the prevention and treatment of coronary artery and arteriolar disease.

Acknowledgments

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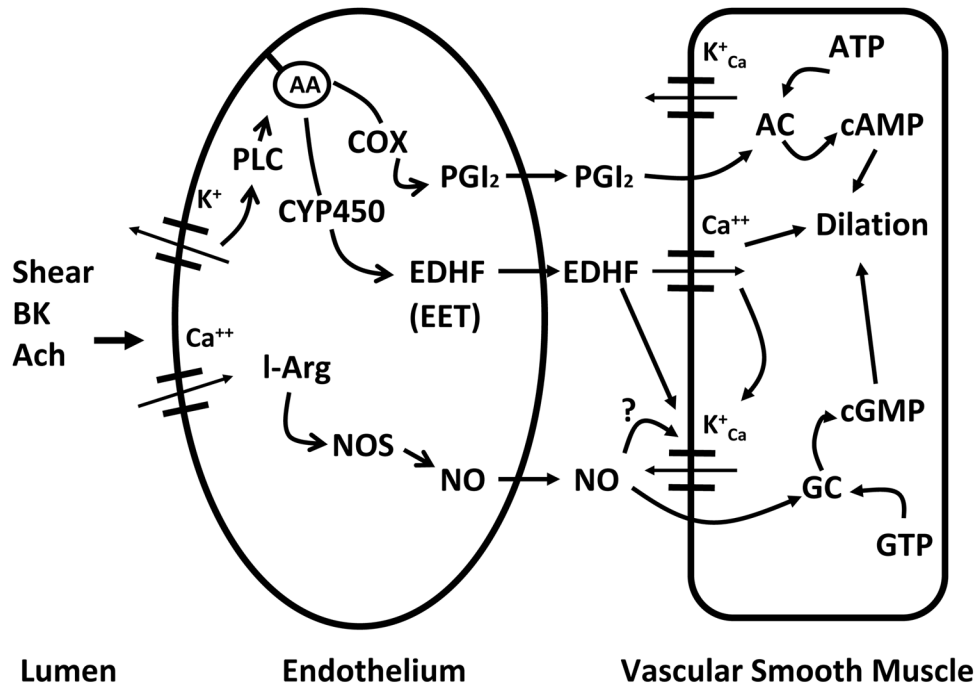
Figure 1.
Traditional endothelium-dependent mediators of vasodilation in the microcirculation.
Agonists such as bradykinin (BK) and acetylcholine (Ach), or mechanical forces such as
shear stress, increase vessel diameter in an endothelium-dependent manner by stimulating
production of nitric oxide, or release of arachidonic acid which then is metabolized to
prostacyclin or epoxyeicosatrienoic acids. These substances move to the underlying vascular
smooth muscle to elicit relaxation through cGMP, cAMP, and/or membrane
hyperpolarization, respectively. AA (arachidonic acid); AC (adenylyl cyclase); ATP
(adenosine triphosphate); cAMP (cyclic adenosine monophosphate); cGMP (cyclic
guanosine monophosphate); COX (cyclooxygenase); CYP450(cytochrome P450
monooxygenase); EDHF (endothelium derived hyperpolarizing factor); EET
(epoxyeicosatrienoic acid); GC (guanylyl cyclase); GTP (guanosine triphosphate); Kca
(large conductance calcium-activated potassium channel); l-arg (L-arginine); NO (nitric
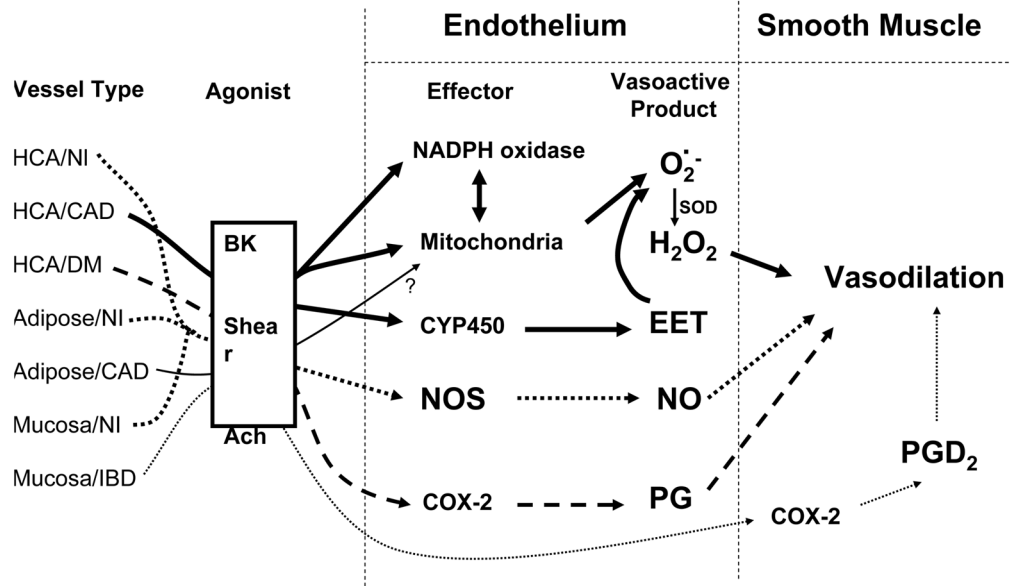oxide); NOS (nitric oxide synthase); PGI2 (prostacyclin); PLC (phospholipase).

Figure 2.
Diversity in endothelium- dependent dilator pathways in the human microcirculation.
Depending on the stimulus/agonist applied and the vessel origin, a variety of pathway
mediators, including NO, prostanoids, reactive oxygen species, and cytochrome P450
metabolites, contribute to endothelium-dependent dilation. Abbreviations similar to Figure
1. CAD (coronary artery disease); DM (diabetes); H_2O_2 (hydrogen peroxide); HCA (human
coronary arterioles); IBD (inflammatory bowel disease); Nl (normal); PG (prostaglandin);
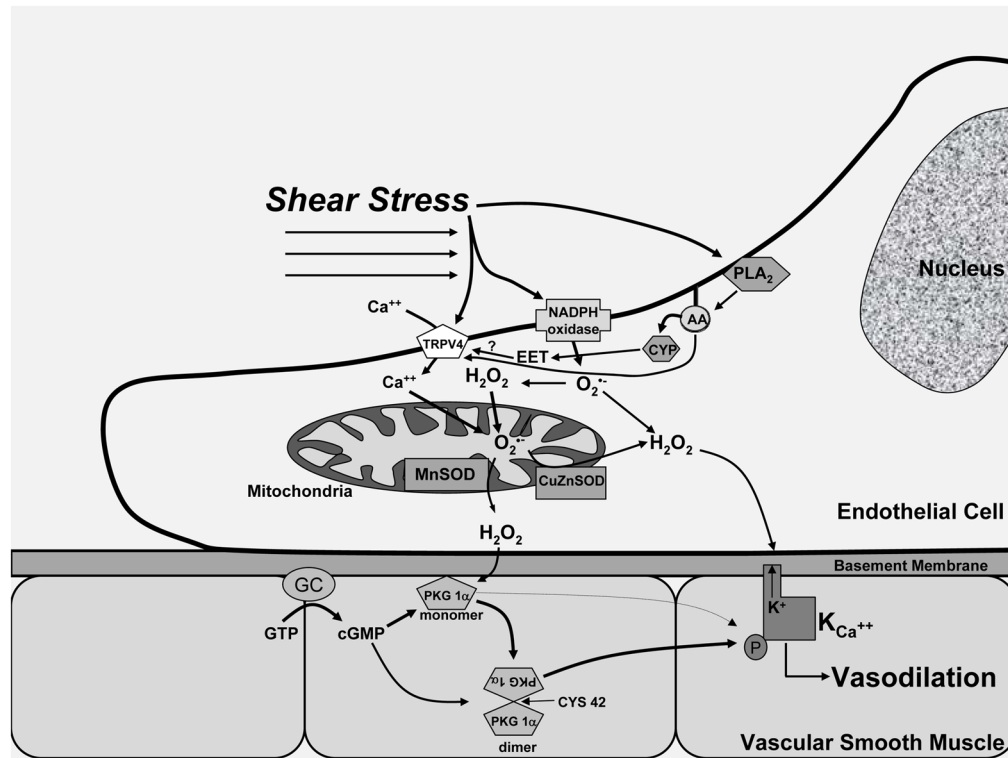PGD2 (prostaglandin D2); SOD (superoxide dismutase).

Figure 3.
Schematic depiction of mechanochemical signaling pathway responsible for flow-mediated dilation in human coronary arterioles. Shear stress can activate phospholipases to cleave arachidonic acid from cell membranes. This serves as a substrate for CYP450 monooxygenase production of EETs. These metabolites and/or shear directly activate TPRV4 channels to enhance calcium entry into endothelial cells. The rise in calcium, together with enhanced production of ROS from NADPH oxidase and mechanosensitive factors (unpublished data), stimulates mitochondrial production of superoxide. The mitochondrial-produced superoxide is dismutated to hydrogen peroxide, which diffuses to the vascular smooth muscle cell layer to oxidize cysteine residues of PKG1α, causing homodimerization and activation of the enzyme. The activated dimer opens BK channels on the cell membrane, leading to hyperpolarization and relaxation of the vascular smooth muscle layer. Abbreviations similar to Figure 1. Also: CuZn SOD (copper-zinc superoxide dismutase); MnSOD (Manganese superoxide dismutase); PKG1α (protein kinase G 1α); PLA$_2$ (phospholipase A$_2$); TPRV4 (transient receptor potential vannilloid 4).